The effect of fluoridated toothpastes on plaque covered enamel in vivo

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SUMMARY

The development of dental caries is influenced by numerous aetiological factors which **together** determine the net effect: whether or not there will be loss of tooth substance. The acid production as a result of bacterial fermentation of carbohydrates in the dental plaque is one factor influencing the caries process. The composition of the tooth mineral, which affects the resistance of the tooth, and of the saliva, together with dietary habits influence caries progression.
Dental caries might be prevented by interfering with one or more of these factors. Removal of the dental plaque by means of good oral hygiene is a very important and effective caries preventive measure, familiar to most people today. Furthermore, decreasing the availability of substrate (especially carbohydrates) essential for the acid producing bacteria is also helpful and reduces dental decay. Probably the most practised measure used to lower the rate of dental disease is, however, the use of fluoride in toothpastes.

The main aim of the investigations in this thesis was to study and compare the effects of the use of several fluoridated toothpaste systems on partially demineralized human enamel in vivo under plaque.
The specific aims of the investigations are:
1) to study the influence of the toothpaste **composition** on efficiency;
2) to study the influence of toothpaste **consumption** on efficiency and
3) to compare the SIMS and microdrill techniques for fluoride analysis.

Dental enamel, which mainly consists of the mineral hydroxy-

apatite is susceptible to mineral loss (demineralization). Under favourable circumstances mineral can also be accumulated (remineralization). In vivo, demineralization and remineralization periods alternate many times every day, each time inducing complex mineral changes. Fluoride influences both de- and remineralization most importantly with respect to:

1. Reduction of enamel solubility;
2. Stimulation of mineral accumulation;
3. Inhibition of microbiological activity in plaque.

A brief review of relevant information from the literature on enamel, caries and remineralization is given in **chapter two.**

This information is mainly based on the results of in vitro studies. Many of these studies were carried out under more or less favourable circumstances and were aimed at demonstrating the beneficial effects of cariostatic agents **under those circumstances.** Furthermore, very few aetiological factors can be studied simultaneously in vitro.

Despite the often very valuble information obtained from in vitro studies information on the development of caries and the effects of cariostatic agents on the caries process in vivo under severe cariogenic challenges is still not complete. Continued research aimed at elucidating these aspects is still needed.

The experimental procedures used in the investigations (chapters 3, 4, 5 and 6) were as follows:

Five toothpastes with different fluoride systems were compared with a placebo control. The fluoride contents of the toothpastes were: A = 1000 ppm F as Na_2PO_3F + 450 ppm F as NaF; B = 1100 ppm F as NaF; C = 1000 ppm F as Na_2PO_3F; D = 1000 ppm F as SnF_2; E = 1500 ppm F as Na_2PO_3F; and P = 0 ppm F (placebo).

Two slabs of flattened human enamel demineralized in vitro in a lactate-MHDP solution were placed in proximal positions in prostheses specially made for each of the participants. The specimens were constantly covered with plaque, since in

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their interproximal position the plaque could not be removed by toothbrushing. The experiments described were therefore carried out under very severe cariogenic test conditions.
Each treatment involved the use of an unfluoridated toothpaste for one week, followed by two weeks of twice-daily use of the test paste. Each participant used all six toothpastes following a randomized block test design.

The determination of the total fluoride uptake in partially demineralized enamel from in situ brushing with the six toothpaste systems is described in **chapter 3.**
After in vitro demineralization the average fluoride content of the enamel specimens was about 2 $\mu g.cm^{-2}$. This level increased to 4.3 $\mu g.cm^{-2}$ fluoride in the specimens treated with the unfluoridated toothpaste, whereas the mean fluoride content of the enamel after use of the fluoridated toothpastes varied from 7.4 - 11.4 $\mu g.cm^{-2}$.
The fluoride uptakes from pastes A, C, D and E were not significantly different from each other but were all significantly higher than the placebo. Statistical analysis showed a significantly higher fluoride level from the NaF-based paste B than from all other systems. There were no differences between mesial-distal specimens and no correlation was found between fluoride uptake and patient age. Significant uptake differences were found between participants with full and partial prostheses, most problably because of differences in the composition of the oral microflora, etc. between these two groups of participants.

A review of fluoride analysis techniques is given in **chapter 4.1.** Briefly described there are: the acid etching technique, in which thin layers of enamel are etched off and the fluoride content in the solutions then determined; the micro-abrasion technique, in which enamel layers are removed by gently grinding; the microdrill technique, in which enamel powder is sampled after drilling a tiny hole in the enamel; and physical techniques, particularly secondary ion mass spectroscopy (SIMS).

A comparison between the SIMS and the microdrill fluoride analysis techniques is made.
Although the concentrations found with the microdrill technique are consistently lower than the corresponding SIMS data, the results obtained using the microdrill technique agree quite well with the SIMS measurements. For routine quantitative fluoride level analyses of enamel, the microdrill approach is both reliable and accurate. The SIMS method, on the other hand, provides a fluoride analysis which enables detailed fluoride level profiles to be determined in enamel. Clearly both techniques are valuable in the analysis of fluoride uptake by partially demineralized enamel.
A comparison of the microdrill fluoride analysis technique and the "KOH extraction method" is given in **chapter 4.3.** The KOH extraction method is used to measure the amounts of loosely bound "calciumfluoride-like" material on the enamel (F_{on}), whereas the microdrill fluoride analysis technique measures the fluoride content in the enamel (F_{in}). It can be concluded that, in **all** cases roughly 45% of the fluoride acquired from toothpastes is deposited as "calciumfluoride-like" material.

An important parameter in this study is the **amount of toothpaste used,** because in reality this controls the amount of fluoride in contact with the teeth.
In **chapter 5** the fluoride uptake in partially demineralized enamel has been correlated with the volume of toothpaste used. The amounts of free ionic fluoride, fluoride as MFP and total fluoride delivered by the toothpastes were measured and correlated with the fluoride uptake observed in the enamel in vivo.
Substantial differences **between** participants were discovered in the volume of toothpaste used daily, the average being about 1.5 cm^3. There was only a relatively small variation in the amount of toothpaste used by each participant for the various pastes tested. In this study, there was a significant correlation ($p<0.05$) between the volumes of fluoride toothpaste used and fluoride uptake, whereas for the non-

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fluoridated toothpaste a correlation coefficient r=0 was found. Because of the limited range of volumes of toothpastes used in this study, some care must be taken when extrapolating these results for (much) lower or higher usage values or to toothpastes with much higher fluoride contents. The level of free ionic fluoride and, to a lesser extent, the level of monofluorophosphate (MFP) **in** the toothpastes were linearly correlated with the fluoride uptake. On the other hand no such correlation was observed for the total amount of fluoride in the toothpastes.

During carious lesion formation the mechanical properties of the enamel change. In this model study the microhardness changes in partially demineralized human enamel were, therefore, studied after the use of the fluoridated toothpastes **in vivo**. The Knoop and Vickers microhardness measurements on sound and on **in vitro** demineralized enamel, before and after **in vivo** exposure of the enamel to the toothpaste treatments are discussed in **chapter 6.**
The results show that the initial indentation lengths of sound enamel (Knoop ≃67 µm, Vickers ≃53 µm) were increased about threefold after the **in vitro** demineralization procedure. After the **in vivo** experiments a further increase in the mean indentation lengths was measured, using the Knoop technique, to ≃300 µm for all six toothpaste systems, including the non-fluoridated toothpaste. The Vickers indentation length values, measuring at a greater depth, remained at the same level as before the experiments.
No correlation could be found between fluoride uptake and changes in microhardness values.
From the microhardness results it can be concluded that despite the availability of fluoride, no net rehardening occurred. This result was probably caused by the fact that the enamel was subjected to severe conditions under plaque in the proximal situation. The relatively short testing period (2 weeks) might also be partially responsible for the failure to observe rehardening.
The results furthermore show the importance of regular pla-

que removal at approximal sites in the clinical situation in order to allow mechanisms associated with remineralization, rather than demineralization, to dominate.

In the general discussion (**chapter 7**) the use of the in situ model designed to promote constant plaque coverage of the enamel specimens is presented. The advantages and disadvantages of the use of flattened in vitro demineralized enamel specimens are discussed.
Although enamel lesions thus prepared are not identical to natural lesions, they are reproducible and very suitable for comparing the efficiency of cariostatic agents (toothpastes). They also allow the use of the microdrill biopsy technique and microhardness measurements.
The role of plaque and especially of calcium fluoride formed during and immediately after fluoridation on (and in or near) the enamel is discussed.
In spite of constant plaque coverage it was shown in our experiments that the fluoride content of the partially demineralized enamel specimens was significantly increased. Plaque is therefore not an important diffusion barrier for fluoride.

Caries is the net result of alternating periods of de- and remineralization; fluoride being beneficial for both processes. In our study using the "constant plaque coverage-model", demineralization predominated and remineralization under plaque was most probably small or negligible.
In **in vitro** experiments methane hydroxy diphosphonate (MHDP) has been shown to inhibit both the de- and remineralization of enamel. **In vivo**, MHDP-containing lesions behave different, because remineralization of MHDP-containing lesions has recently been reported in vivo.
These results on the remineralization of MHDP-lesions are important because they show that both the demineralization process (dominating in this work) as well as the remineralization process are **not** controlled by the presence of MHDP in vivo.

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Therefore, the results of this study are probably applicable to lesion progress (and the influence of fluoride) in general.

A most important conclusion from this work is that fluoride from toothpastes can be acquired in substantial amounts by demineralized enamel **despite constant plaque coverage.** The beneficial effects of fluoride on remineralization of enamel lesions under plaque, however, seemed overshadowed by the severe cariogenic activity of the plaque.

It can be concluded from this study that the efficiency of fluoride as a caries preventive measure is to a large extent dependent on local conditions in the mouth. The quantitative effect of fluoride as an inhibitor for the caries process depends very strong on the fact whether a site is intermittently covered with plaque or almost constantly.